Best Available Copy

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 31/185, 31/33, 31/38, 31/40, 31/425, 31/445

A1

(11) International Publication Number: WO 94/20085

(43) International Publication Date: 15 September 1994 (15.09.94)

(21) International Application Number: PCT/US94/01971

(22) International Filing Date: 24 February 1994 (24.02.94)

(30) Priority Data:
08/025,046 1 March 1993 (01.03.93) US
08/168,817 15 December 1993 (15.12.93) US

(71) Applicant: CHILDREN'S HOSPITAL MEDICAL CENTER CORPORATION [US/US]; 300 Longwood Avenue, Boston, MA 02115 (US).

(72) Inventor: D'AMATO, Robert; 911 Forest Road, Lancaster, PA 17601 (US).

(74) Agents: JOHNSON, James, Dean et al.; Jones & Askew, 37th floor, 191 Peachtree Street, N.E., Atlanta, GA 30303-1769 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS

(57) Abstract

The present invention comprises a group of compounds that effectively inhibit angiogenesis. More specifically, thalidomide and various related compounds such as thalidomide precursors, analogs, metabolites and hydrolysis products have been shown to inhibit angiogenesis. Importantly, these compounds can be administered orally.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/025,046, filed March 1, 1993.

Technical Field

The present invention relates to methods and compositions for preventing unwanted angiogenesis in a human or animal. More particularly, the present invention relates to a method for preventing unwanted angiogenesis, particularly in angiogenesis dependent or associated diseases, by administration of compounds such as thalidomide and related compounds.

Background of the Invention

As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals only undergo angiogenesis in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The control of angiogenesis is a highly regulated system of angiogenic stimulators and inhibitors. The control of angiogenesis has been found to be altered in

certain disease states and, in many cases, the pathological damage associated with the disease is related to the uncontrolled angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological states created due to unregulated angiogenesis have been grouped together as angiogenic dependent or angiogenic associated diseases. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

One example of a disease mediated by angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated visual problems are caused by an ingrowth of chorioidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium. Angiogenic damage is

also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phylectenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, mariginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Another disease in which angiogenesis is believed to be involved is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth

and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Factors associated with angiogenesis may also have a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors would promote new bone formation. Therapeutic intervention that prevents the bone destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such disease states as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood vessels into the inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

One of the most frequent angiogenic diseases of childhood is the hemangioma. In most cases, the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, the hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nosebleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

It should be noted that angiogenesis has been associated with blood-born tumors such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.

Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of

microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors.

Angiogenesis is also involved in normal physiological processes such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several kinds of compounds have been used to prevent angiogenesis. Taylor et al. have used protamine to inhibit angiogenesis, see Taylor et al., *Nature* 297:307 (1982). The toxicity of protamine limits its practical use as a therapeutic. Folkman et al. have disclosed the use of heparin and steroids to control angiogenesis. See Folkman et al., *Science* 221:719 (1983) and U.S. Patent Nos. 5,001,116 and 4,994,443. Steroids, such as tetrahydrocortisol, which lack gluco and mineral corticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors such as interferon inhibit angiogenesis. For example, interferon α or human interferon ß has been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon ß is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. See Sidky et al., *Cancer Research* 47:5155-5161 (1987). Human recombinant α interferon (alpha/A) was reported to be successfully used in the treatment of pulmonary hemangiomatosis, an angiogenesis-

induced disease. See White et al., *New England J. Med.* 320:1197-1200 (1989).

Other agents which have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. See Japanese Kokai Tokkyo Koho No. 58-131978. Sulfated polysaccharide DS 4152 also shows angiogenic inhibition. See Japanese Kokai Tokkyo Koho No. 63-119500. A fungal product, fumagillin, is a potent angiostatic agent *in vitro*. The compound is toxic *in vivo*, but a synthetic derivative, AGM 12470, has been used *in vivo* to treat collagen II arthritis. Fumagillin and O-substituted fumagillin derivatives are disclosed in EPO Publication Nos. 0325199A2 and 0357061A1.

PCT Application No. WO 92/14455 to Kaplan et al. is directed to a method for controlling abnormal concentration of TNF-α by administering thalidomide or thalidomide derivatives to a patient with toxic concentrations of TNF-α.

The above compounds are either topical or injectable therapeutics. Therefore, there are drawbacks to their use as a general angiogenic inhibitor and lack adequate potency. For example, in prevention of excessive wound healing, surgery on internal body organs involves incisions in various structures contained within the body cavities. These wounds are not accessible to local applications of angiogenic inhibitors. Local delivery systems also involve frequent dressings which are impracticable for internal wounds, and increase the risk of infection or damage to delicate granulation tissue for surface wounds.

Thus, a method and composition are needed that are capable of inhibiting angiogenesis and which are easily administered. A simple and efficacious method of treatment would be through the oral route. If an angiogenic inhibitor could be given by an oral route, the many kinds of diseases discussed above, and other angiogenic dependent pathologies, could be treated easily. The optimal dosage could be distributed in a form that the patient could self-administer.

Summary of the Invention

In accordance with the present invention, compositions and methods are provided that are effective in inhibiting unwanted angiogenesis. These compositions are easily administered by different routes including oral and can be given in dosages that are safe and provide angiogenic inhibition at internal sites. The present invention provides a method of treating mammalian diseases mediated by undesired and uncontrolled angiogenesis by administering a composition comprising an anti-angiogenic compound in a dosage sufficient to inhibit angiogenesis.

The present invention also includes angiogenic inhibiting compounds that contain an epoxide group. These angiogenic inhibiting compounds can be administered to a human or animal alone or with epoxide hydrolase inhibiting compounds.

The present invention is especially useful for treating certain ocular neovascular diseases such as macular degeneration. The compounds which are contemplated as part of the present invention preferably can be given orally to the patient and thereby halt the progression of the disease. Other disease that can be treated using the present invention are diabetic retinopathy, neovascular glaucoma and retrolental fibroplasia.

Accordingly, it is an object of the present invention to provide a compound and method to inhibit unwanted angiogenesis in a human or animal.

It is yet another object of the present invention to provide a composition of inhibiting angiogenesis by oral administration of the composition.

It is another object of the present invention to provide a treatment for diseases mediated by angiogenesis.

It is yet another object of the present invention to provide a treatment for macular degeneration.

It is yet another object of the present invention to provide a treatment for all forms of proliferative

It is yet another object of the present invention to provide a treatment for all forms of proliferative vitreoretinopathy including those forms not associated with diabetes.

It is yet another object of the present invention to provide a treatment for solid tumors.

It is yet another object of the present invention to provide a method and composition for the treatment of blood-born tumors such as leukemia.

It is another object of the present invention to provide a method and composition for the treatment of hemangioma.

It is another object of the present invention to provide a method and composition for the treatment of retrolental fibroplasia.

It is another object of the present invention to provide a method and composition for the treatment of psoriasis.

It is another object of the present invention to provide a method and composition for the treatment of Kaposi's sarcoma.

It is another object of the present invention to provide a method and composition for the treatment of Crohn's diseases.

It is another object of the present invention to provide a method and composition for the treatment of diabetic retinopathy.

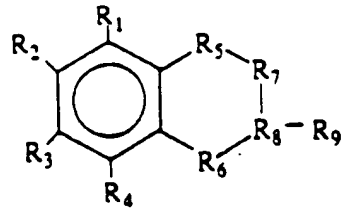
Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

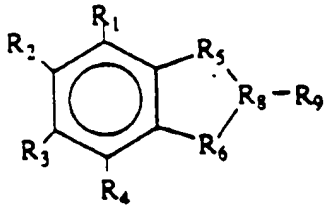
Brief Description of the Figures

Figures 1 through 3 are a listing of representative compounds in the genus represented by the following general formulas:

A)



B)



C)

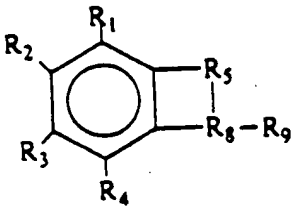


Figure 4 is a listing of representative compounds in the genus represented by the following general formula:

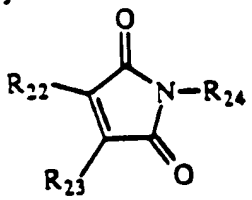


Figure 5 is a listing of representative compounds in the genus represented by the following general formula:

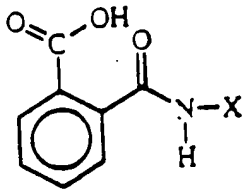


Figure 6 shows the effect of thalidomide and EM12 on angiogenesis in a rabbit cornea model of angiogenesis.

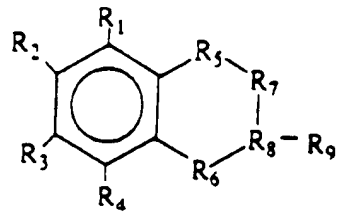
Figure 7 shows the effect of thalidomide on the area of corneal vascularization in a rabbit cornea model of angiogenesis.

Detailed Description

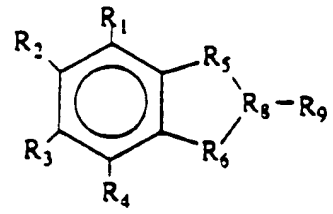
The present invention includes compositions and methods for the treatment of diseases that are mediated by angiogenesis. One embodiment of the present invention is the use of thalidomide or the metabolites of thalidomide as disclosed herein to inhibit unwanted angiogenesis. The present invention also includes compounds which cause dysmelia is the developing fetus and have anti-angiogenic activity. The present invention comprises a method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic.

Compounds that can be used in accordance with the present invention include compounds included in the following general formulae. Examples of compounds that have anti-angiogenic properties having one of the following three formulae (A), (B), or (C):

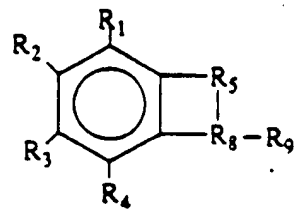
A)



B)



C)



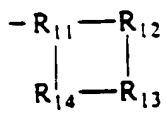
In the above formulae A), B), and C), R_1, R_2, R_3 and R_4 can be selected from: -H; -OH; =O, straight chained and branched alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acid, ester, or ether moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; $-XO_n$ or $-O-XO_n$, [where X=N and n=2; X=S and n=2 or 3; or X=P and n=1-3]; and halogens; R_5, R_6, R_7, and R_8 are each independently selected from:

$$-\overset{Y}{\underset{}{C}}-R_{10};\quad -\overset{Y}{\underset{}{N}}-R_{10};$$

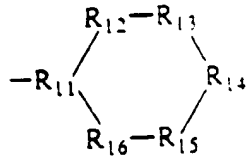
or -O- where Y is optional and is the same as defined above for R_1; and R_{10} is the same as defined above for R_1, or (where Y is

absent) R10 is =O; and R9 is a moiety having formula D), E), F), G) or H):

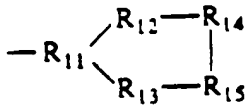
D)



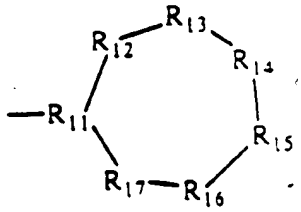
F)



E)

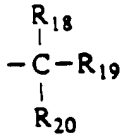


G)

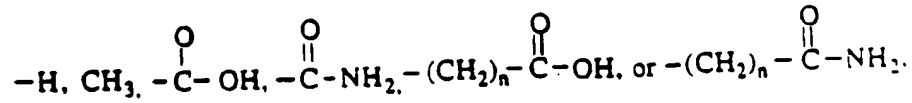


where each of R11 - R17 is (independently) the same as defined above for R5;

H)



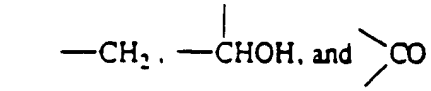
where R18, R19 and R20 are, independently selected from



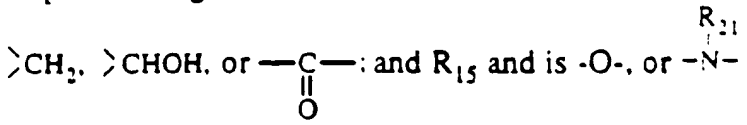
and n=1 to 4.

Accordingly, another aspect of the present invention features inhibiting angiogenesis in a mammal by administering a therapeutic composition comprising one of the above-described compounds in a dosage sufficient to inhibit angiogenesis

In preferred embodiments, the compound has formula B), where R_5 and R_6 are selected from the group consisting of:

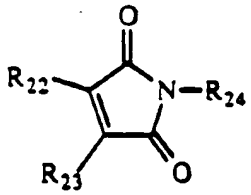


and R9 has formula F) or H); and R_{14} and R_{16} are selected from the group consisting of:



where R_{21} is -H, $-CH_3$, or -OH. Specific preferred compounds according to this aspect of the present invention include thalidomide, its precursors, metabolites and analogs. Particular analogs include EM-12, N-phthaloyl-DL-glutamic acid (PGA) or N-phthaloyl-DL-glutamine anhydride. Examples of compounds that are members of this genus are listed in Figures 1 through 3. It is to be understood that the compounds included as part of the present invention are not to be limited to those compounds shown in Figures 1 through 3 and include all other compounds that are members of the genus described by the general formulas herein.

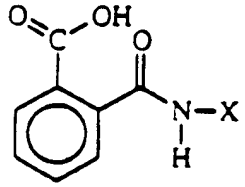
Compounds of the following formula that have anti-angiogenic properties:



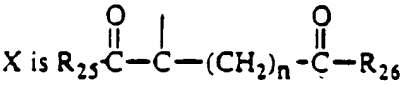
where R_{22} and R_{23} are (independently), -H, -F, -Cl, -Br, -I, $-CH_3$, or $-CH_2-CH_3$; and R_{24} is -H, $-CH_3$, or $-CH_2-CH_3$.

The present invention also features inhibiting angiogenesis in a mammal by administering a compound according to the above formulae in a dosage sufficient to inhibit angiogenesis. Examples of specific compounds that are members of this genus are listed in Figure 4.

Angiogenesis inhibition hydrolysis products of thalidomide having the following general formula can be used in practicing the present invention:

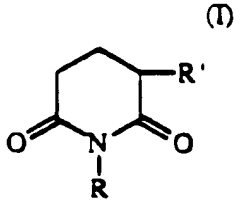


where X is R_6 as defined above, or

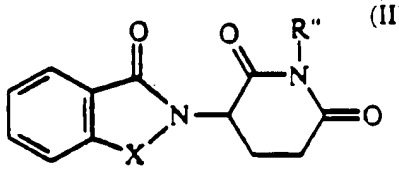


and R_{25} and R_{26} are, independently, -OH, -H, or NH_2, and n=1 through 4. Examples of such compounds are shown in Figure 5.

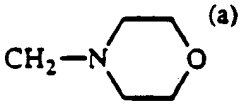
Angiogenesis inhibition compounds having the following general formula can be used in practicing the present invention:



(I)



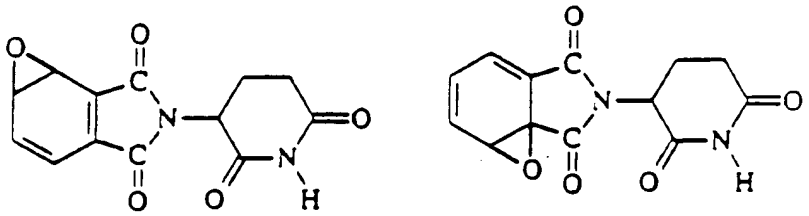
(II)



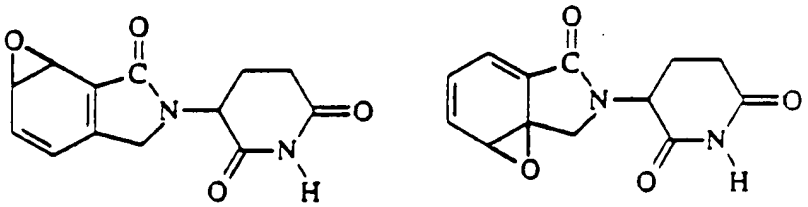
(a)

wherein compounds of structure (I), wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical and of structure (II), wherein X is CH_2 or C=O; R" is H, $-CH_2CH_3$, $-C_6H_5$, $-CH_2C_6H_5$, $-CH_2CH=CH_2$, or (a) and hydrolysis products of the compounds wherein R" is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

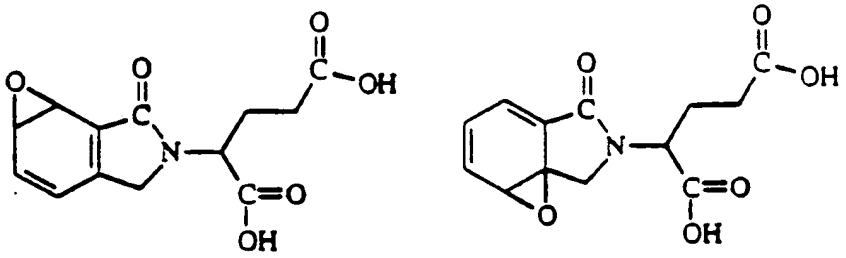
Another set of compounds that are considered part of the present invention are the epoxides of thalidomide, EM-12 and EM-138. Representative epoxide compounds are shown as follows:



Epoxides of thalidomide



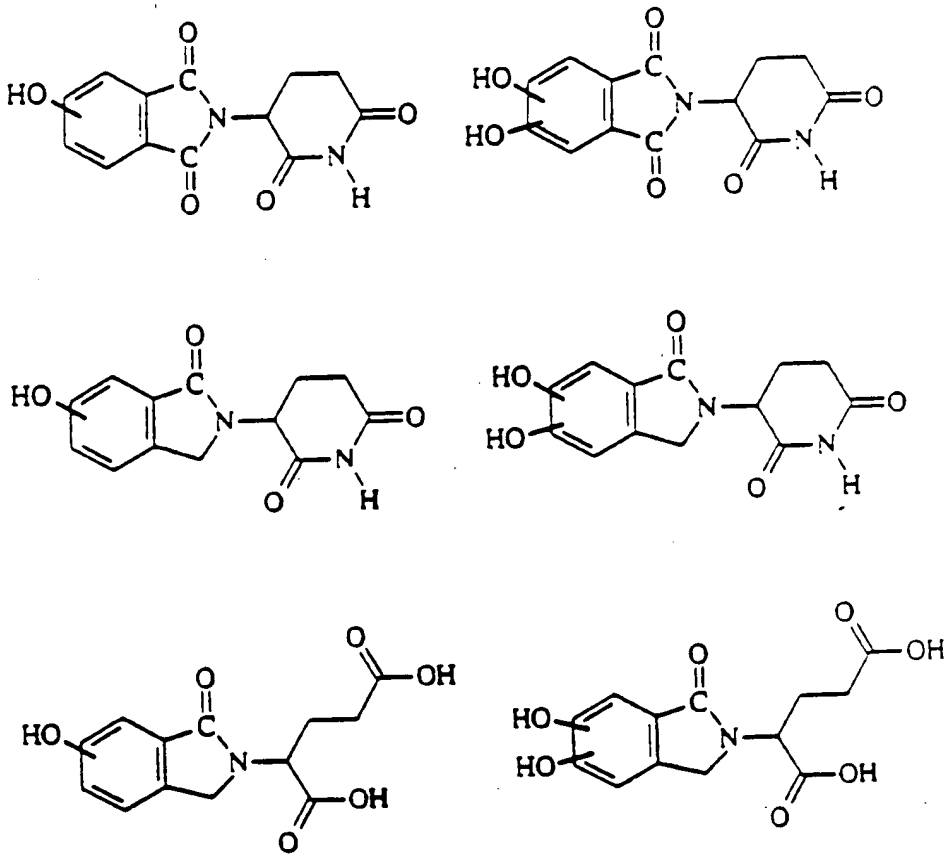
Epoxides of EM 12



Epoxides of EM 138

It should be understood that the epoxide can be attached at the 6,1 site on the benzene ring, the 1,2 site, the 2,3 site 3,4 or the 4,5 site. All of these compounds are contemplated as part of the present invention.

The epoxides of the thalidomide, EM-12, and EM 138 can be hydrolized to the following compounds:



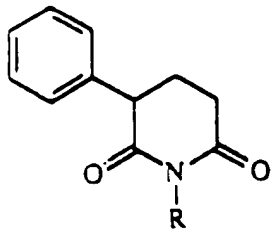
It is to be understood that the hydroxyl group can be on carbons 1, 2, 3, 4, 5 and 6 of the benzene ring. Also contemplated as part of the present invention are dihydroxyl compounds wherein the two hydroxyl groups are located bis to each other on carbons 1, 2, 3, 5 and 6 of the above compounds. The epoxides, the hydrolysis products of the epoxides, and the hydrolysis products of the thalidomide are all contemplated to be part of the present invention.

It is known that epoxides are hydrolized by a group of enzymes known as epoxide hydrolases. There is a class of compounds which are epoxide hydrolase inhibitors. Examples of these compounds are valpromide (2-propylpentanamide) and valproic acid (2-propylpentanoic acid). Because epoxides are

important angiogenesis inhibitors, it is contemplated as part of the present invention, compositions comprising any of the angiogenesis inhibitors compounds recited herein in combination with epoxide hydrolase inhibitors. The epoxide hydrolase inhibitors can be administered to a human or animal together or sequentially. The expoxide group appears to be an important substituent common to several angiogenesis inhibitors. The use of epoxide hydrolase inhibitors to potentiate the activity of any angiogenesis inhibitor containing an epoxide is contemplated as part of the present invention. For example, the epoxide hydrolase inhibitors can be administered with the following epoxide-containing anti-angiogenesis compounds: AGM 1470, Eponimycin, microbial metabolites of *Scolecobasidium arenarium* designated f/2015, fr/111142 and fr/18487. See Oikawa, *Biochem Biophys. Res. Comm*, Vol. 81:1070 (1971) and Otsuka, *J. Microbial. Biotech.*, Vol 1:163 (1991).

It is contemplated as an embodiment of the present invention the use of the epoxide containing angiogenesis inhibitors with or without epoxide hydrolase inhibitors as a treatment for diseases mediated by elevated or toxic levels of TNF-α. TNF-α has been recognized as manifesting a dose dependent toxicity. If present at low levels for a long period of time, TNF-α can result in cachexia. Cachexia is a general weight loss and wasting occurring in the course of some chronic diseases such as cancer, opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, and high dose IL-2 therapy. The epoxide containing angiogenesis inhibitors, with or without epoxide hydrolase inhibitors, are also effective in treating diseases such as septic shock, leprosy and graph vs. host disease.

Other embodiments are within the present invention. For example, other dysmelia-causing compounds can be used according to the present invention, e.g. 4-methylphthalic acid, pyridoxine, vasopressin, acetazolamide, or a compound having the following formula (where R= H, -OH, or $-CH_3$):



Other compounds which are teratogens, such as valproic acid (2-propylpentanoic acid), the retinoids, such as cis-retinoic acid, and rifampin may also be used in accordance with the invention.

In summary, the preferred compounds are thalidomide, as well as analogs, hydrolysis products, metabolites and precursors of thalidomide that are teratogenic, and, more specifically, that cause dismelia. However, it is to be understood that it is not necessary for a compound to have both teratogenic activity and angiogenesis inhibiting activity to be considered part of the present invention. Dysmelia-causing compounds can be identified by the general procedures of Helm, *Arzneimittle-forschung*, 31(i/6):941-949 (1981), in which rabbit pups are examined after exposure to the compound in utero. The compounds can generally be purchased, e.g., from Andrulis Pharmaceuticals, Beltsville, MD, or synthesized according to known procedures. It is to be understood that the compounds of the present invention can exist as enantiomers and that the racemic mixture of enantiomers or the isolated enantiomers are all considered as within the scope of the present invention.

Many of the compounds that are contemplated as part of the present invention can be enriched in optically active enantiomers of the compounds specified above. Specifically, *Blaschke* has reported that the S enanantiomers may be disproportionately responsible for the dismelia-producing effect of these compounds. See, generally Blaschke, *Arzneimittelforschung* 29:1640-1642 (1979). The above described articles generally describe procedures to obtain optically active preparations of the compounds of interest. See, e.g. Shealy et al., *Chem. Indus.* 1030 (1965); and Casini et al.,

Farmaco Ed. Sci. 19:563 (1964).

The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor. The biodegradable polymers and their use are described, for example, in detail in Brem et al., *J. Neurosurg.* 74:441-446 (1991).

The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use. For oral administration to humans, a dosage of between approximately 0.1 to 300 mg/kg/day, preferably between approximately 0.5 and 50 mg/kg/day, and most preferably between approximately 1 to 10 mg/kg/day, is generally sufficient.

The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracameral) nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into associate the active ingredient with liquid

carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tamports, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of formulation in question, for

example, those suitable for oral administration may include flavoring agents.

Diseases associated with corneal neovascularization that can be treated according to the present invention include but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phylectenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, mariginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

Diseases associated with retinal/choroidal neovascularization that can be treated according to the present invention include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Another disease which can be treated according to the present invention is rheumatoid arthritis. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Another disease that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example I

The chick embryo chorioallantoic membrane assay described by Crum et al., *Science* 230:1375 et seq. (1985), is used to identify compounds that do not require further metabolic conversion. See also, U.S. Patent 5,001,116, hereby incorporated by reference, which describes the CAM assay at col. 7 of the patent. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the compound is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured.

Example II

Rabbit cornea angiogenesis assay

Pellets for implantation into rabbit corneas were made by mixing 110 µl of saline containing 12 µg of recombinant bFGF (Takeda Pharmaceuticals-Japan) with 40 mg of sucralfate (Bukh Meditec-Denmark); this suspension was added to 80 µl of 12% hydron (Interferon Sciences) in ethanol. 10 µl aliquots of this mixture was then pipetted onto teflon pegs and allowed to dry producing approximately 17 pellets. A pellet was implanted into corneal micropockets of each eye of an anesthetized female New Zealand white rabbit, 2mm from the limbus followed by topical application of erythromycin ointment onto the surface of the cornea. The animals were fed daily from 2 days post-implantation by gastric lavage with either drug suspended in 0.5% carboxymethyl cellulose or 0.5% carboxymethyl cellulose alone. Thalidomide was purchased from Andrulus Pharmaceutical (Maryland) and the EM-12 and Supidimide were kindly provided by Grunenthal GMBH (Germany). The animals were examined with a slit lamp every other day in a masked manner by the same corneal specialist. The area of corneal neovascularization was determined by measuring with a reticule the vessel length (L) from the limbus and the number of clock hours (C) of limbus involved. A formula was used to determine the area of a circular band segment: $C/12 * 3.1416 [r^2-(r-L)^2]$ where r=6 mm the measured radius of the rabbit cornea. Various mathematical models were utilized to determine the amount of vascularized cornea and this formula was found to provide the most accurate approximation of the area of the band of neovascularization that grows towards the pellet.

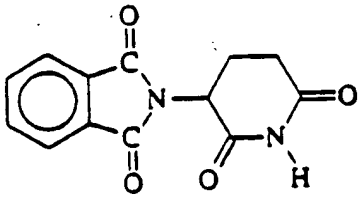
It is important to note that the rabbit cornea assay is preferable because it will generally recognize compounds that are inactive *per se* but are metabolized to yield active compounds. Thalidomide related compounds, as shown below in Example III. are known to be teratogens and are candidates for use in the present invention.

Example III

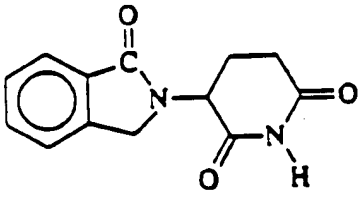
Inhibition of bFGF induced corneal neovascularization by thalidomide and related analog expressed as percent of median control on day 8

Pellets containing bFGF and sucralfate were implanted into micropockets of both corneas of rabbits according to Example II. Vessel ingrowth into clear cornea from the limbus was first noted on day 2 and treatments (200 mg/kg orally) were begun on this day. The area of corneal neovascularization was measured from day 4 through day 12. Day 8 measurements were used for comparison between groups. No regression of vessels and near maximal neovascularization was seen at this time point. Statistical analysis was performed with ANOVA with ranked data to account for interexperimental variation and to guard against a non-normal distribution of data (i.e. outliers) by utilizing a nonparametric method.

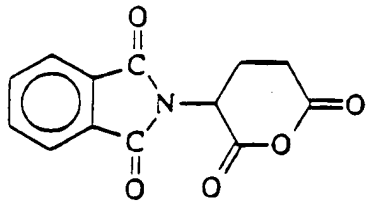
The compounds tested were as follows:



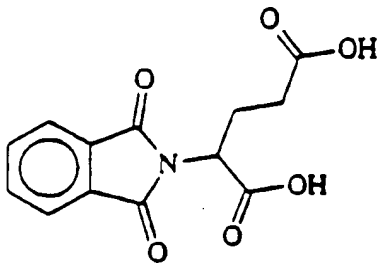
thalidomide



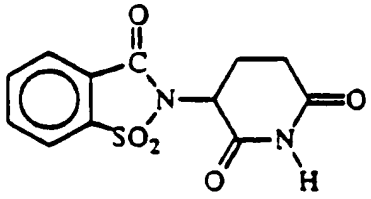
EM-12



phthaloyl glutamic anhydride (PGA)



phthaloyl glutamic acid (PG Acid)



supidimide.

Treatment with a dose of (200 mg/kg) of thalidomide resulted in an inhibition of the area of vascularized cornea that ranged from 30-51% in three experiments with a median inhibition of 36% (Figure 6) (n=30 eyes, p=0.0001, 2 way ANOVA with ranked data). The inhibition of angiogenesis by thalidomide was seen after only two doses (Figure 7). The rabbits did not demonstrate obvious sedation and there were no signs of toxicity or weight loss. The teratogenic analog EM-12, which shares the other properties of thalidomide was also inhibitory, with a median inhibition of 42% (n=10 eyes, p=0.002, 1-way ANOVA with ranked data). Supidimide, a nonteratogenic analog of thalidomide that retains the sedative properties of thalidomide, exhibited no activity (area 107% of control, n=10 eyes, not statistically different from control). Other analogs, PGA and PG acid displayed weaker inhibitory effects than thalidomide (data

not shown). The density of vessel ingrowth in thalidomide-treated animals was also markedly reduced.

Example IV

EM-12 in rabbit cornea assay

EM-12 was tested in the rabbit cornea assay described in Example II at 100 mg/kg/day and showed 21% inhibition, and at 200mg/kg/day the assay showed 43% inhibition.

Example V

Phthaloyl glutamic acid in CAM

Phthaloyl glutamic acid was tested in the above described CAM assay and exhibit an avascular zone with a mild scar.

Example VI

Phthaloyl glutamic acid in rabbit cornea assay

Phthaloyl glutamic acid described above at 200 mg/kg and exhibited 29% inhibition of angiogenesis.

Example VII

Phthaloyl glutamic anhydride in CAM assay

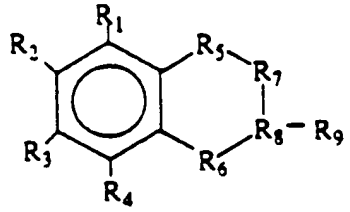
Phthaloyl glutamic anhydride was test in the CAM assay described above and exhibited an avascular zone.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

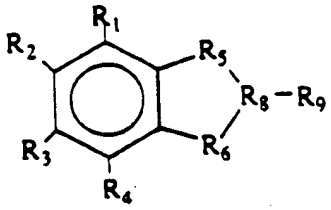
CLAIMS

1. A method of treating undesired angiogenesis in a human or animal comprising the step of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis-inhibiting compound selected from the group consisting of the following compounds:

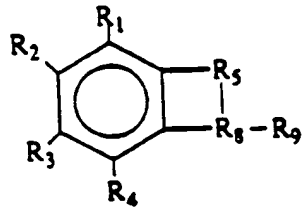
A)



B)

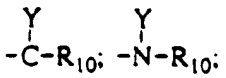


C)



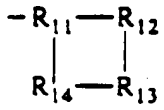
In the above formulae A), B), and C), R_1, R_2, R_3 and R_4 can be selected from: -H; -OH; =O, straight chained and branched alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acid, ester, or ether moieties in combination with acyclic, cyclic, or combination

acyclic/cyclic moieties; aza; amino; $-XO_n$ or $-O-XO_n$, [where X=N and n=2; X=S and n=2 or 3; or X=P and n=1-3]; and halogens; R_5, R_6, R_7, and R_8 are each independently selected from:

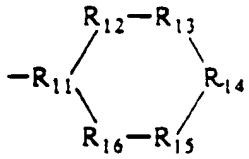


or -O- where Y is optional and is the same as defined above for R_1; and R_{10} is the same as defined above for R_1, or (where Y is absent) R_{10} is =O; and R9 is a moiety having formula D), E), F), G) or H):

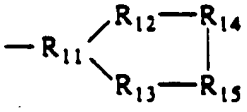
D)



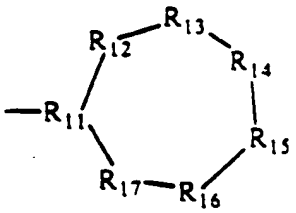
F)



E)

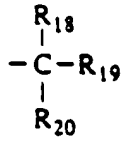


G)



where each of R_{11} - R_{17} is (independently) the same as defined above for R_5;

H)

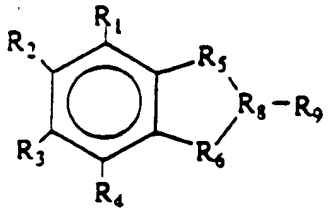


where R_{18}, R_{19} and R_{20} are, independently selected from

$-H$, CH_3, $-\overset{O}{\overset{\|}{C}}-OH$, $-\overset{O}{\overset{\|}{C}}-NH_2$, $-(CH_2)_n-\overset{O}{\overset{\|}{C}}-OH$, or $-(CH_2)_n-\overset{O}{\overset{\|}{C}}-NH_2$.

and n=1 to 4.

2. The method of claim 1, wherein the compound has the following formula:



and R5 and R6 are selected from the group consisting of

$-CH_2-$, $-\overset{|}{C}HOH$, and $>C\!=\!O$

and in which R9 has formula F) or H), and R_{14} and R_{16} are selected from the group consisting of,

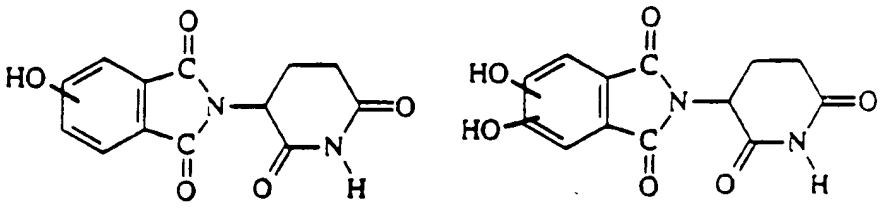
$>CH_2$, $>CHOH$, or $-\overset{O}{\overset{\|}{C}}-$; and R_{15} and is -O-, or $-\overset{R_{21}}{\overset{|}{N}}-$,

where R_{21} is -H, $-CH_3$, or -OH.

3. The method of claim 1, wherein the compound is thalidomide.

4. The method of claim 1, wherein the compound is a thalidomide metabolite or hydrolysis product.

5. The method of Claim 1, wherein the compound is selected from the group consisting of the following compounds:



6. The method of claim 1, wherein the compound is selected from the group consisting of N-phthaloyl-DL-glutamic acid (PGA) and N-phthaloyl-DL-glutamine anhydride.

7. The method of claim 1; wherein the compound is EM-12.

8. The method of claim 1, wherein the compound is selected from the group of compounds shown in Figures 1 through 3.

9. The method of Claim 1, wherein the composition further comprises an epoxide hydrolase inhibitor.

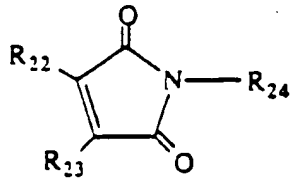
10. The method of Claim 1, wherein the undesired angiogenesis is associated with retinal/choroidal neovascularization.

11. The method of Claim 10, wherein the retinal/choroidal newvascularization is associated with diabetic retinopathy.

12. The method of Claim 10, wherein the retinal/choroidal newvascularization is associated with macular degeneration.

13. The method of Claim 1, wherein the undesired angiogenesis is associated with corneal neovascularization.

14. A method of treating undesired angiogenesis in an human or animal comprising the step of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis-inhibiting compound selected from the group consisting of the following compounds:

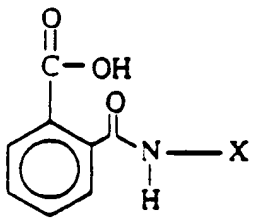


where R_{22} and R_{23} are (independently), -H, -F, -Cl, -Br, -I, $-CH_3$, or $-CH_2-CH_3$; and R_{24} is -H, -OH, $-CH_3$, or $-CH_2-CH_3$.

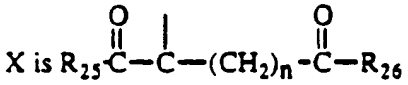
15. The method of Claim 14, wherein the composition further comprises an epoxide hydrolase inhibitor.

16. The method of claim 14, wherein the compound is selected from the group of compounds shown in Figure 4.

17. A method of treating undesired angiogenesis in a human or animal comprising the step of administering to the human or animal with the undesired angiogenesis an effective amount of a compound selected from the group consisting of the following compounds:



where X is R_6 as defined above, or X is

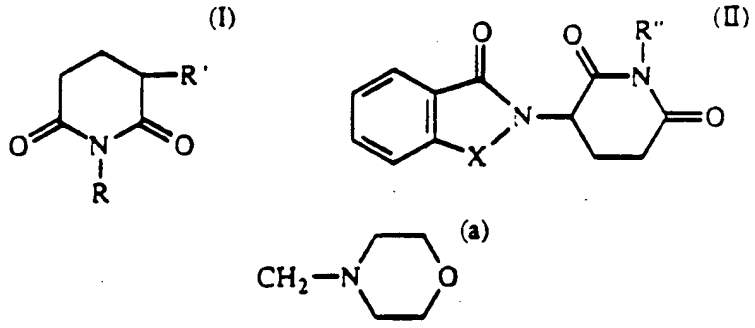


and R_{25} and R_{26} are, independently, -OH, -H, or $-NH_2$.

18. The method of claim 17, wherein the compound is selected from the group consisting of the compounds in Figure 5.

19. The method of Claim 17, wherein the composition further comprises an epoxide hydrolase inhibitor.

20. A method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis-inhibiting compound selected from the group consisting of the following compounds:



wherein compounds of structure (1), wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical and of structure (II), wherein X is CH_2 or C=O; R" is H, $-CH_2CH_3$, $-C_6H_5$, $-CH_2C_6H_5$, $-CH_2CH=CH_2$, or structure (a) and hydrolysis products of the compounds wherein R" is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

21. A method of treating undesired angiogenesis in a human or animal comprising the step of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic in a pharmaceutically acceptable carrier.

22. A method of treating undesired angiogenesis in a human or animal comprising the step of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis inhibitor containing an epoxide group and and effective amount of an epoxide hydrolase inhibitor.

23. A method of treating a human or animal that has toxic concentrations of TNF-α comprising the step of administering to the human or animal that has toxic concentrations of TNF-α an effective amount of an angiogenesis inhibitor containing an epoxide group.

24. The method of Claim 24, wherein the composition further contains an effective amount of an epoxide hydrolase inhibitor.

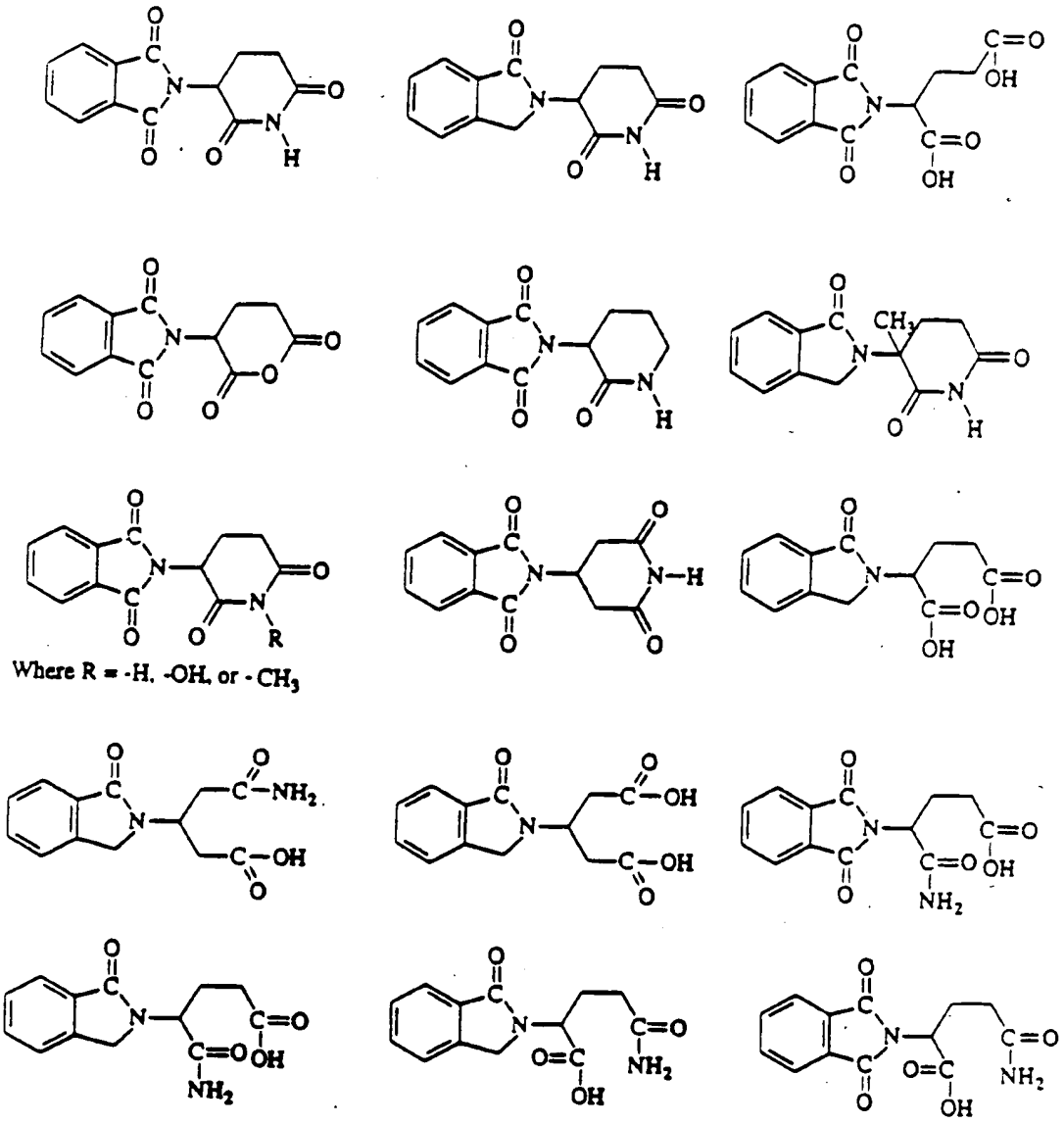


Figure 1

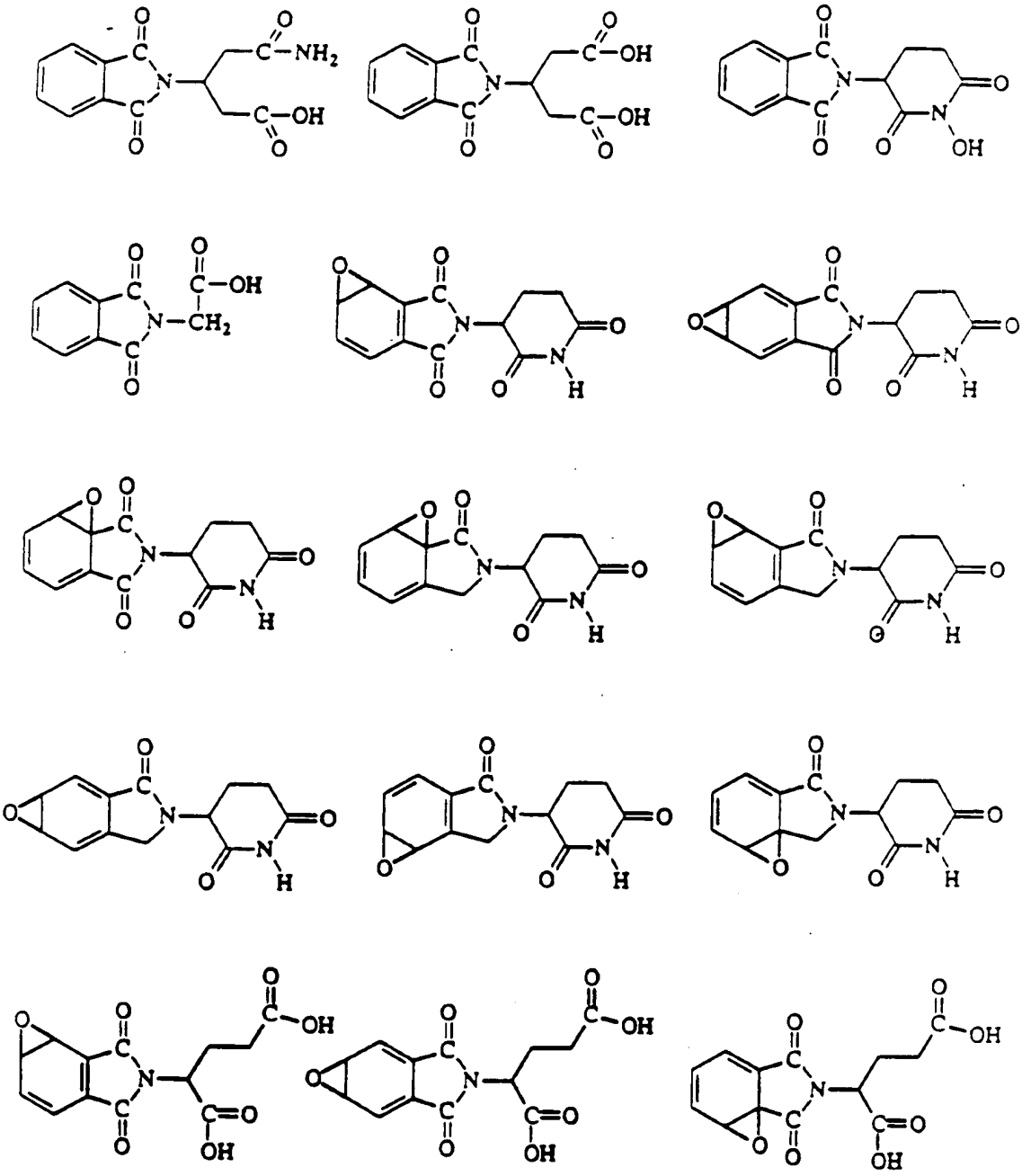


Figure 2

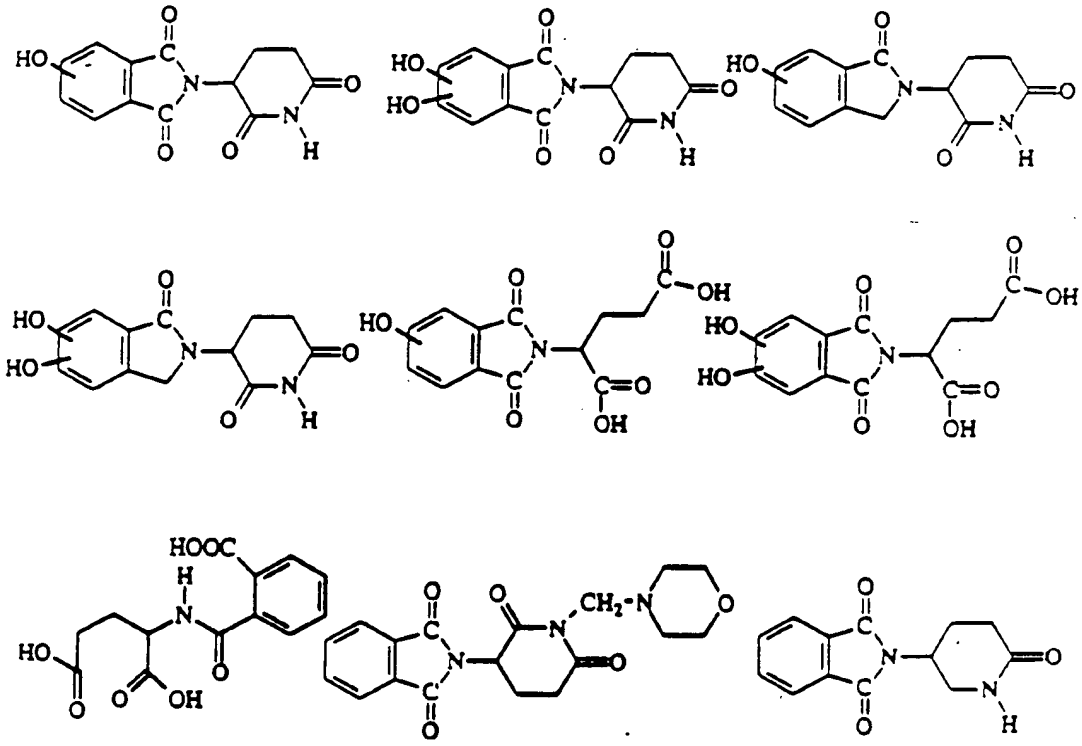


Figure 3

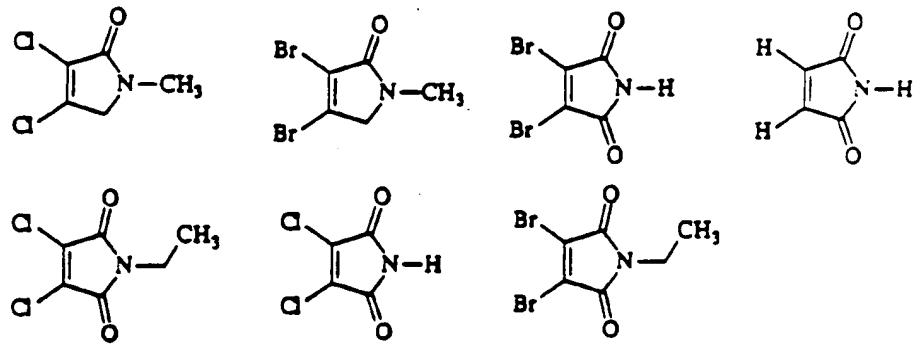


Figure 4

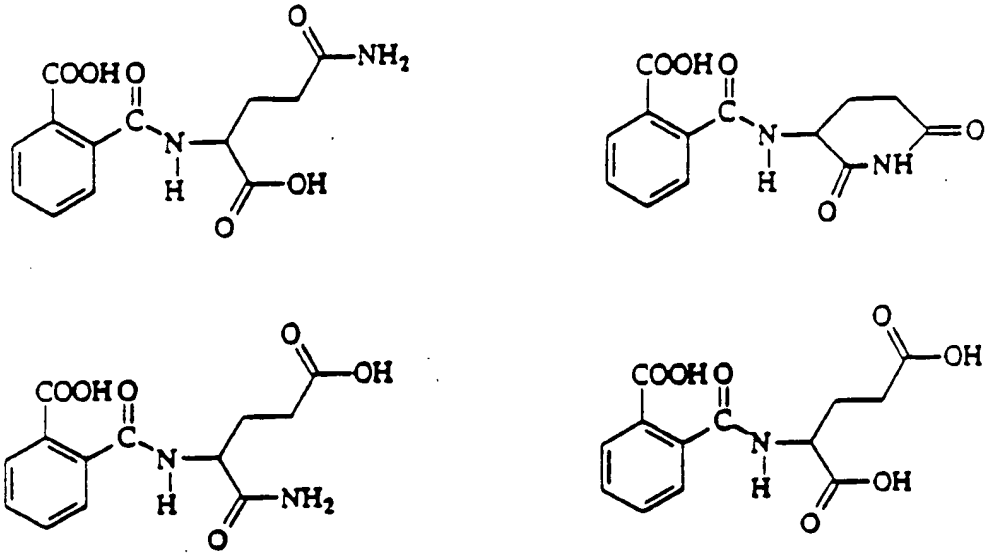


Figure 5

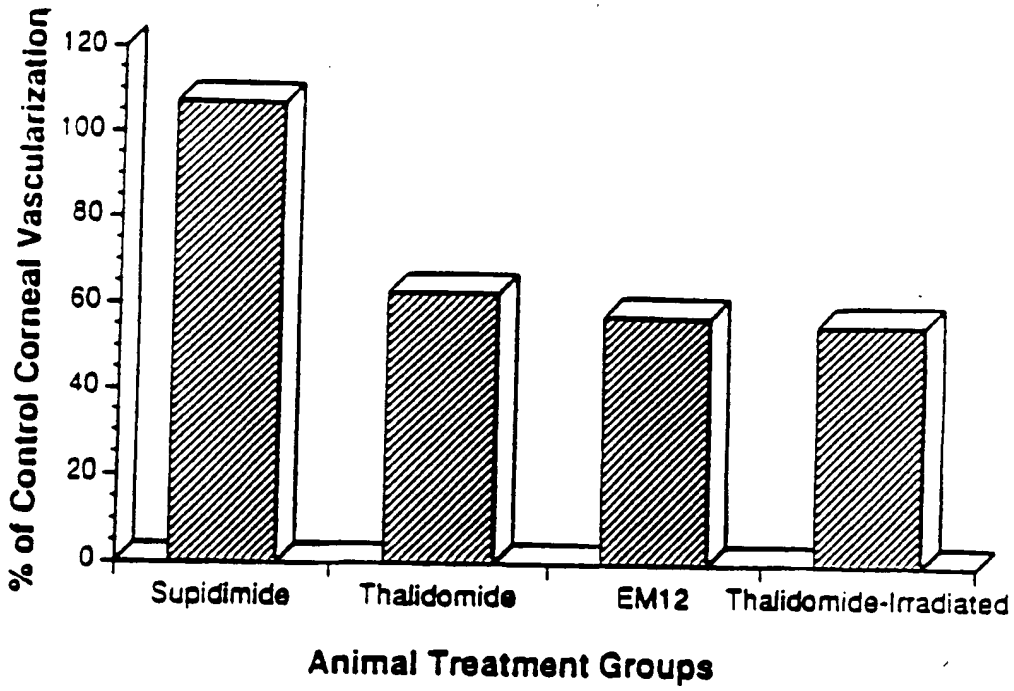


Figure 6

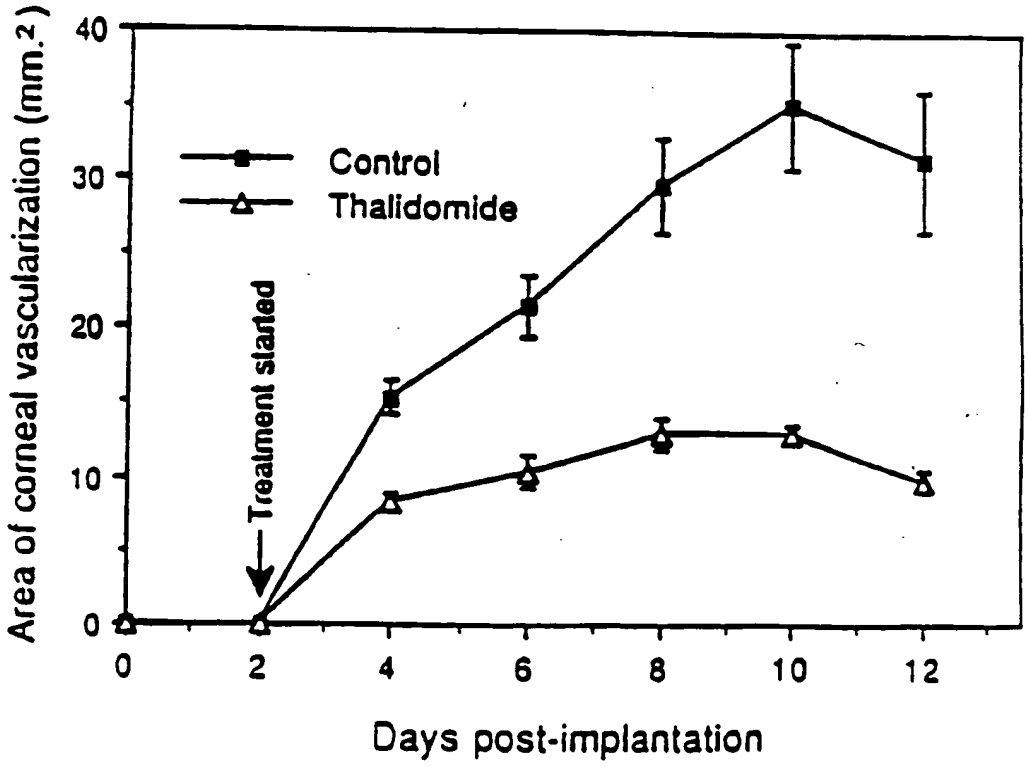


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01971

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/185, 31/33, 31/38, 31/40, 31/425, 31/445

US CL :514/210, 338, 373, 411, 414, 416, 417, 553, 559

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/210, 338, 373, 411, 414, 416, 417, 553, 559

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A 5,021,404 (FOLKMAN ET AL) 04 JUNE 1991, see entire document.	1-13
A	US, A 4,552,88 (KOPPEL ET AL) 12 NOVEMBER 1985, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 MAY 1994	JUN 02 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN PEABODY Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/US94/01971

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-23 (to extent below)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see attached sheets

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01971

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

The claims are seen to involve a multitude of inventions (see Box II) so diverse that a search of all inventions would require an inordinate amount of time. Therefore, the inventions are searched to the extent that they cover those in Group I of the Lack of Unity, below, and other specifically named compounds at pages 15, 16, 17, 26, and 27 of the description, claims 3,5,6,7, and 16 (Figure4).

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING:

Group 1. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is four membered and includes at least one nitrogen classified in class/subclass 514/210.

Group 2. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is seven-membered and includes at least on hetero atom other than nitrogen, classified in class/subclass 514/211.

Group 3. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is seven membered consisting of one nitrogen and six carbon atoms, classified in class/subclass 514/212.

Group 4. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is seven-membered consisting of two nitrogens and five carbon atoms, classified in class/subclass 514/218.

Group 5. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein there are there or more ring heteroatoms in the six-membered ring, classified in class/subclass 514/229.2

Group 6. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein there is a polycyclo ring system having the six-membered hetero ring as on of the cycles, wherein said polycyclo is a bicyclo ring system, classified in class/subclass 514/230.5.

Group 7. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein there is an oxygen atom bonded directly to a ring carbon of a 1,4-oxazine ring, classified in class/subclass 514/230.8.

Group 8. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein the hetero ring is a morpholine having -C(=O)- bonded directly to the morpholine ring, classified in class/subclass 514/237.5.

Group 9. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein the hetero ring is a morpholine having nitrogen attached indirectly to the morpholine ring by acyclic aomomic bonding, classified in class/subclass 514/237.8.

Group 10. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein the hetero ring is a morpholine having oxygen attached indirectly to the morpholine ring by acyclic aomomic bonding, classified in class/subclass 514/238.8.

Group 11. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein the hetero ring is a morpholine having a carbocyclic ring attached indirectly to the morpholine ring by acyclic aomomic bonding, classified in class/subclass 514/239.5.

Group 12. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, wherein the hetero ring is a morpholine and not encompassed by the previous groups, classified in class/subclass 514/233.1.

Group 13. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered and includes at least nitrogen and oxygen as ring hetero atoms, classified in class/subclass 514/228.8.

Group 14. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of three nitrogens and three carbon atoms, where in the triazine is asymmetrical and not part of a polycyclo ring system, classified in class/subclass 514/242.

Group 15. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of three nitrogens and three carbon atoms, where in the triazine is asymmetrical and part of a polycyclo ring system, classified in class/subclass 514/243.

Group 16. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of three nitrogens and three carbon atoms, wherein a nitrogen atom is bonded directly to the ring carbon of the hetero ring, classified in class/subclass 514/245.

Group 17. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of three nitrogens and three carbon atoms and not encompassed by the previous groups, classified in class/subclass 514/241.

Group 18. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a polycyclo ring system having a 1,2-diazine as one of the cycles, classified in class/subclass 514/248.

Group 19. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a polycyclo ring system having a 1,4-diazine as one of the cycles, classified in class/subclass 514/249.

Group 20. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a polycyclo ring system having a hetero ring as part of the polycyclo ring system other than a 1,2- or 1,4-diazine , classified in class/subclass 514/254.

Group 21. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,4-diazine, classified in class/subclass 514/255.

Group 22. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine that is part of a bicyclo ring system and is a quinazoline having nitrogen bonded directly to the 1,3-diazine at the 2 position, classified in class/subclass 514/260.

Group 23. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine that is part of a bicyclo ring system and is a quinazoline not encompassed by the

previous group, classified in class/subclass 514/259.

Group 24. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine that is part of a bicyclo ring system and not encompassed by the previous groups, classified in class/subclass 514/258.

Group 25. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine that is a pyrimidine with a oxygen bonded directly to the pyrimidine ring, classified in class/subclass 514/269.

Group 26. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine having nitrogen bonded directly to the 1,3-diazine a the 2-position by a single bond, classified in class/subclass 514/269.

Group 27. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms wherein there is a 1,3-diazine not encompassed by the previous groups, classified in class/subclass 514/256.

Group 28. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of two nitrogens and four carbon atoms and not encompassed by the previous groups, classified in class/subclass 514/247.

Group 29. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and having plural hetero atoms in the bicyclo ring system and wherein there is oxygen in the bicyclo ring system, classified in class/subclass 514/302.

Group 30. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and having plural hetero atoms in the bicyclo ring system and wherein there are exactly three ring nitrogens in the ring, classified in class/subclass 514/303.

Group 31. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and having plural hetero atoms in the bicyclo ring system and not encompassed by the previous groups, classified in class/subclass 514/300.

Group 32. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is isoquinoline and having oxygen attached directly to the six-membered hetero ring, classified in class/subclass 514/309.

Group 33. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is isoquinoline and having nitrogen, other than as nitro or nitroso, attached directly to the six-membered hetero ring, classified in class/subclass 514/310.

Group 34. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is isoquinoline and not encompassed by the previous groups, classified in class/subclass 514/307.

Group 35. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is quinoline and having oxygen attached directly to the six-membered hetero ring, classified in class/subclass 514/312.

Group 36. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is quinoline and having nitrogen, other than as nitro or nitroso, attached directly to the six-membered hetero ring, classified in class/subclass 514/313.

Group 37. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is quinoline and having an addition hetero ring attached directly or indirectly to the quinoline ring system, classified in class/subclass 514/314.

Group 38. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and is quinoline and not encompassed by the previous groups, classified in class/subclass 514/311.

Group 39. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms that is part of a bicyclo ring system and not encompassed by the previous groups, classified in class/subclass 514/299.

Group 40. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring, wherein oxygen and nitrogen are both bonded to the same carbon, classified in class/subclass 514/346.

Group 41. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring wherein oxygens are bonded directly to at least two ring carbons of the six-membered hetero ring, classified in class/subclass 514/348.

Group 42. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring and having nitrogen bonded directly to the six-membered hetero ring, classified in class/subclass 514/349.

Group 43. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring and having C=O bonded directly to the six-membered hetero ring, classified in class/subclass 514/350.

Group 44. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring and having nitrogen bonded indirectly to the six-membered hetero ring, classified in class/subclass 514/351.

Group 45. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having oxygen bonded directly to ring carbon of the six membered hetero ring and not encompassed by the previous groups, classified in class/subclass 514/345.

Group 46. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having nitrogen bonded directly to ring carbon of the six membered hetero ring and not encompassed by the previous groups, classified in class/subclass 514/352.

Group 47. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine having C=O bonded directly to ring carbon of the six membered hetero ring and not encompassed by the previous groups, classified in class/subclass 514/354.

Group 48. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen

and five carbon atoms and is a piperidine having nitrogen bonded indirectly to ring carbon of the six membered hetero ring and not encompassed by the previous groups, classified in class/subclass 514/357.

Group 49. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and is a piperidine not encompassed by the previous groups, classified in class/subclass 514/315.

Group 50. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is six-membered consisting of one nitrogen and five carbon atoms and not encompassed by the previous groups, classified in class/subclass 514/377.

Group 51. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and having plural ring oxygens, classified in class/subclass 514/360.

Group 52. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an oxadiazole, classified in class/subclass 514/364.

Group 53. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-oxazole having a polycyclo ring system that contains the hetero ring, classified in class/subclass 514/375.

Group 54. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-oxazole having an oxygen atom bonded directly to the hetero ring, classified in class/subclass 514/376.

Group 55. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-oxazole having a nitrogen atom bonded directly to the hetero ring, classified in class/subclass 514/377.

Group 56. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-oxazole and not encompassed by the previous groups, classified in class/subclass 514/374.

Group 57. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-oxazole having a polycyclo ring system that contains the hetero ring, classified in class/subclass 514/379.

Group 58. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-oxazole having an oxygen atom or a nitrogen atom bonded directly to the hetero ring, classified in class/subclass 514/380.

Group 59. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-oxazole and not encompassed by the previous groups, classified in class/subclass 514/378.

Group 60. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is a part of a polycyclo ring system which is benzofused at the 4,5-positions and not encompassed by the previous groupings, classified in class/subclass 514/394.

Group 61. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is a part of a polycyclo ring system which is benzofused at the 4,5-positions and has oxygen or nitrogen atoms bonded directly at the 1,2 or 3 position of the diazole ring, classified in class/subclass 514/395.

Group 62. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is a part of a polycyclo ring system and not encompassed by the previous groupings, classified in class/subclass 514/393.

Group 63. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is an imidazole having oxygen or nitrogen bonded directly to the hetero ring, classified in class/subclass 514/398.

Group 64. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is an imidazole having oxygen or nitrogen bonded indirectly to the hetero ring, classified in class/subclass 514/399.

Group 65. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is an imidazole and not encompassed by the previous groupings, classified in class/subclass 514/396.

Group 66. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole which is an imidazoline, classified in class/subclass 514/401.

Group 67. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,3-diazole and not encompassed by the previous groups, classified in class/subclass 514/385.

Group 68. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-diazole wherein there is a divalent oxygen bonded directly the hetero ring which is part of polycyclo ring system, classified in class/subclass 514/405.

Group 69. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-diazole wherein there is a divalent oxygen bonded directly the hetero ring and not encompassed by the previous groups, classified in class/subclass 514/404.

Group 70. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-diazole is a pyrazole wherein oxygen or nitrogen bonded directly to the hetero ring, classified in class/subclass 514/407.

Group 71. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-diazole is a pyrazole and not encompassed by the previous groups, classified in class/subclass 514/406.

Group 72. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and wherein the hetero ring is an 1,2-diazole and not encompassed by the previous groups, classified in class/subclass 514/403.

Group 73. The method of using compounds according to claims 1-13 and 20-24, wherein the hetero ring is five-membered ring containing at least one nitrogen ring atom and consists of one nitrogen and four carbon atoms, wherein the hetero ring is part of a polycyclo ring system.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☑ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.